Prostate Specific Antigen
AIA-PACK PA

Caution: The sale and distribution of this device is restricted by United States federal law to, by, or on the order of a physician. In addition, the use of this device is restricted to, by, or on the order of a physician. Because of differences in reagent specificity and assay methods, the concentration of PSA in a given specimen may vary with devices from different manufacturers. Values obtained with different assay methods cannot be used interchangeably. It is mandatory that results reported by the laboratory to the physician include the identity of the assay used. If the assay method for PSA is changed during the course of monitoring patients with serial PSA levels, baseline values for the patients being serially monitored must be confirmed by additional sequential testing.

Name and Intended Use

AIA-PACK PA is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of Prostate Specific Antigen (PSA) in human serum on TOSOH AIA System analyzers. This device is indicated for the measurement of serum PSA in conjunction with Digital Rectal Examination (DRE) as an aid in the detection of prostate cancer (CaP) in men fifty years of age and older. Prostate biopsy is required for the diagnosis of cancer.

This device is further indicated for the serial measurement of PSA in human serum to be used as an aid in the management of patients with prostatic cancer.

Summary and Explanation of Test

Prostate Specific Antigen (PSA) was identified in 1977 by Wang, et al.[1] PSA is a single chain glycoprotein of approximately 34,000 daltons containing 7% carbohydrate.[2] Functionally and immunohistochemically PSA is distinct from prostatic acid phosphatase (PAP)[3] and is confined to the cytoplasm of prostatic acinar cells and ductal epithelium.[4]

The presence of PSA has been demonstrated in normal, benign hyperplastic, and malignant prostatic tissue, in metastatic prostatic carcinoma, and also in prostatic fluid as well as seminal plasma.[5] PSA is not present, however, in any other tissue from men, nor is it produced by cancers of the lung, colon, rectum, stomach, pancreas or thyroid.[6] Elevated serum PSA levels have been reported in patients with prostate cancer, benign prostatic hypertrophy, or inflammatory conditions of other adjacent genitourinary tissues.[3,7] Prostate cancer cannot be diagnosed until biopsy results confirm the presence of cancer cells. Studies indicate that PSA is an important tool in assessing the effect of therapy.[8] Especially in patients being treated with hormone therapy, concurrent serial determinations of PSA and PAP may provide added clinical value in monitoring patients with prostatic cancer.[9]

Digital rectal examination (DRE) is a widely used technique for detecting prostate cancer, but this procedure used by itself can miss significant numbers of prostatic cancers especially organ confined tumors presenting in prostate locations difficult to palpate. The detection of these organ confined tumors is particularly important since an effective means of treatment of tumors found at this early stage currently exists. In addition, it has been clearly demonstrated that the incidence of prostate cancer increases with age. With the increases in life expectancy, it is even more critical that we be able to diagnose the disease early because more men will develop prostate cancer during their lifetime.

Since the mid-1980's, there has been a growing body of literature concerning the utility of Prostate Specific Antigen (PSA) for both monitoring and detection of prostate cancer (CaP). Catalona et al. evaluated the detection of prostate cancer in conjunction with DRE. Of the 6,630 men participating in the study, biopsies were performed on 1,167 men for a biopsy rate of 17.6%. To be eligible for biopsy in this study required a PSA value greater than 4.0 ng/mL or a suspicious DRE result. Prostate cancer was found in 264 of these patients (22.6%) a percentage that is lower than more recent studies involving serially accrued patients undergoing biopsy procedures. The general finding was that PSA enhances the ability of DRE and the detection of prostate cancer when used in combination.

Principle of the Assay

The AIA-PACK PA is a two-site immunoenzymometric assay which is performed entirely in the AIA-PACK. PSA present in the test sample is bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labeled monoclonal antibody in the AIA-PACK. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the PSA concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve. Due to the epitope sites used, the Tosoh AIA-PACK PA (PSA) has been shown to react in an equimolar fashion with both unbound PSA (free PSA) and that complexed to anti-chymotrypsin (PSA-ACT).

Material Provided (AIA-PACK PA, Cat. No. 020263)

10 trays x 20 test cups (AIA-PACK PA Test Cup)

Plastic test cups containing lyophilized magnetic beads coated with anti-PSA mouse monoclonal antibody and mouse monoclonal antibody (to human PSA) conjugated to bovine alkaline phosphatase with 0.1% sodium azide as a preservative.

Materials Required But Not Provided

The following materials are not provided but are required to perform Prostate Specific Antigen analysis using the AIA-PACK PA (Cat. No. 020263) on the TOSOH AIA Systems. They are available separately from TOSOH.

Materials				Cat. No.
AIA Nex·IA				018539
AIA Nex·IA (TLA)				018540
AIA-PACK				
Substrate Set II				020968
Substrate/Reconstituent				
PA Calibrator Set				020363
Calibrator	Zero	0	ng/mL	
	Positive	50	ng/mL (approx.)	
PA Sample Diluting Solution				020563
Wash Concentrate Set				020955
Diluent Concentrate Set				020956
Detector Standardization Test Cups				020970
Sample Treatment Cups				020971
AIA Nex·IA Sample Cups				018581
AIA Nex·IA Pipette Tips				018552
AIA Nex·IA Preloaded Pipette Tips				018583

Only materials obtained from TOSOH should be used. Materials obtained elsewhere should not be substituted since assay performance is based strictly on TOSOH materials.

Warnings and Precautions

- The AIA-PACK PA is intended for in vitro diagnostic use only.
- Test cups from different lots should not be mixed within a tray.
- AIA-PACK PA contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Human sera is not used in the preparation of this product, however, since human specimens will be used for samples and other quality control products in the lab may be derived from human serum, please use standard laboratory safety procedures in handling all specimens and controls.
- Do not use beyond the expiration date.
- The AIA-PACK PA has been designed so that the high dose "hook effect" is not a problem for the vast majority of samples. Samples with PSA concentrations between 100 and 10,000 ng/mL will read > 100 ng/mL. The "hook effect" phenomenon may occur only at PSA concentrations > 10,000 ng/mL.

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at the specified temperature.

Materials	Cat. No.
Refrigerator Temperature (2° - 8° C):	
AIA-PACK PA	020263
Calibrator Set	020363
Sample Diluting Solution	020563
Calibration Verification/Linearity Test Set	020663
Substrate Set II	020968
Wash Concentrate	020955
Diluent Concentrate	020956
Room Temperature (18° - 25° C):	
Detector Standardization Test Cups	020970
Sample Treatment Cups	020971

AIA-PACK test cups may be stored in the sorter drawer for up to 72 hours at a room temperature of 18° - 25° C. Calibrators must be kept tightly sealed and refrigerated at 2° - 8° C. After opening, calibrators should be used within 24 hours. After opening, Sample Diluting Solution is stable for up to 90 days refrigerated at 2° - 8° C. Reconstituted substrate solution is stable for 3 days at room temperature (18° - 25° C) or 7 days in the refrigerator (2° - 8° C). Working diluent and wash solutions are stable for 30 days at room temperature (18° - 25° C). Reagents should not be used if they appear cloudy or discolored.

Specimen Collection and Handling

Serum is required for the assay. EDTA and citrated plasma SHOULD NOT BE USED.

No special patient preparation is necessary. A venous blood sample is collected aseptically without additives. Store at room temperature until a clot has formed (usually 15-45 minutes), then centrifuge to obtain the serum specimen for assay.

Because there is controversy as to whether any prostatic manipulation affects the PSA results, samples should be drawn before any prostatic procedures such as DRE, TRUS, prostatic massage and TRUS are performed.

Samples may be stored at 2° - 8° C for up to 24 hours prior to analysis. If the analysis cannot be done within 24 hours, the sample should be stored frozen at -20° C or below for up to 60 days.

Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter should be centrifuged prior to testing. Prior to assay, slowly bring frozen samples to room temperature (18° - 25° C) and mix gently.

The sample required for analysis is 20 µL.

Procedure

I. Reagent Preparation

A. Substrate Solution

Bring all reagents to room temperature (18° - 25° C) before preparing the working reagent. Add the entire contents of the Substrate Reconstituent (100 mL) to the lyophilized Substrate; mix thoroughly to dissolve.

B. Wash Solution

Add the entire contents of the Wash Concentrate (100 mL) to approximately 2.0 L of CAP Class I or NCCLS Type I Reagent Grade water, mix well, and adjust the final volume to 2.5 L.

C. Diluent

Add the entire contents of the Diluent Concentrate (100 mL) to approximately 4.0 L of CAP Class I or NCCLS Type I Reagent Grade water, mix well, and adjust the final volume to 5.0 L.

II. Calibration

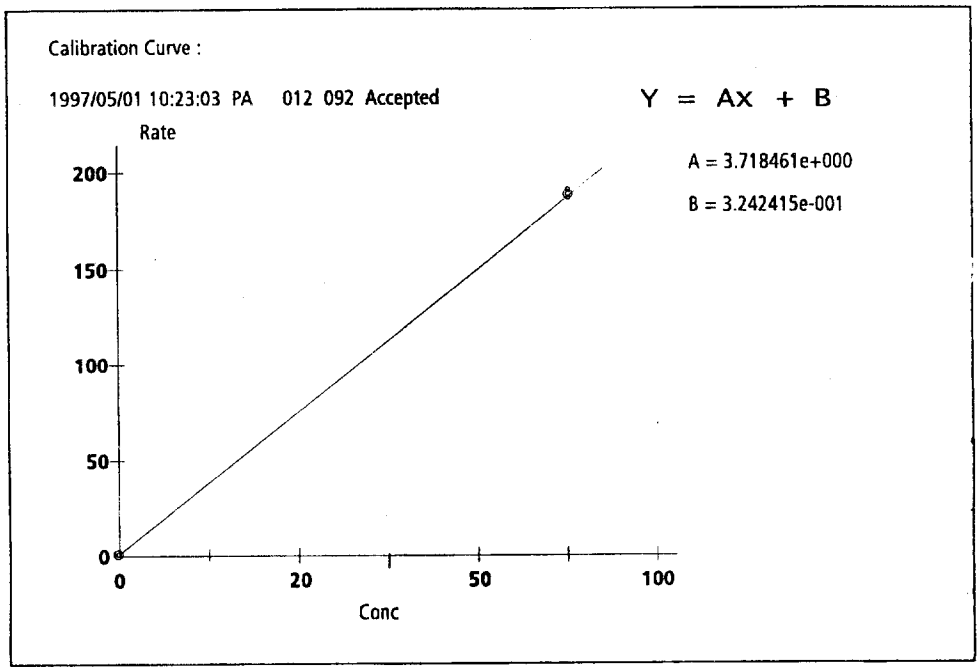
A. Calibration Curve

The calibrators for use with the AIA-PACK PA are prepared gravimetrically and are compared to internal reference standards.

The calibration curve for the AIA-PACK PA is stable for up to 90 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and AIA System maintenance according to the manufacturer's instructions.

Recalibration may be necessary more frequently if controls are out of the established range for this assay or if certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, or detector lamp adjustment or change). For further information regarding instrument operation, consult the AIA System Operator's Manual.

A sample calibration curve from the AIA Nex·IA follows and shows the algorithm used for calculating results.



B. Calibration Procedure

1. Place AIA-PACK PA test cup trays in the sorter drawer.
2. From the Main Menu, select CALIBRATION. Select TEST FILE and verify that the calibrator lot number and concentrations are correct. If not, first update the 16-digit Calibrator Lot, and then the calibrator concentrations.
3. To schedule a calibration, select CALIBRATION REQUEST. Click on Calibrator Lot and enter the 16-digit calibrator set lot number.
4. From the Calibration Start screen, print a worklist. Following the sequence of the worklist, pipette sufficient calibrator into sample cups and place in holders in the sample rack. When operating in the barcode mode, the sample cups are barcoded. Follow instructions for assaying in the barcode mode. When operating in non-barcode mode, place the sample rack in the carousel in the positions shown on the calibration worklist.
5. Select START. Verify that the carousel positions on the worklist match the starting carousel position on the screen.

C. Calibration Acceptability Criteria

1. The mean rate for the zero calibrator should be < 3.0 nM/sec.
2. Since there is a direct relationship between concentration and rate, the rates should increase as the concentration increases.
3. The replicate values should be within a 10% range.

D. Calibration Review and Acceptance

1. From the Main Menu, select CALIBRATION, and then CALIBRATION REVIEW.
2. Select the Pending calibration from the Calibration Curve list box. Calculate and review the data. Accept the calibration.

Cal check controls may be defined to validate the calibration prior to accepting. For further information regarding calibration, consult the AIA System Operator's Manual.

III. Quality Control

A. Commercially Available Controls

Commercially available controls should be run at least once per day. It is recommended that at least two (2) levels of controls, normal and abnormal, be used. Laboratory policy for this particular assay designates the following:

Control Material: _____
Frequency: _____

Lot number of control material, acceptable limits, and corrective action to be taken if controls do not meet laboratory criteria will be found in a separate quality control document maintained by the laboratory.

B. Quality Control Procedure

1. Quality Control material files may be defined in SYSTEM UTILITIES, QC ADD/EDIT. Refer to the AIA System Operator's Manual for detailed instructions on defining and editing the files.
2. From SPECIMEN PROCESSING, select ADD CONTROL. Select the QC Material to be assayed. Click in the first available cell in the Analyte column and select the first analyte to be run. Continue until all analytes are scheduled. Repeat for each control to be run.
3. Assay as described below.

IV. Specimen Processing

A. Preparation

Primary tubes are placed directly in the sample rack. Sample cups are placed in sample cup holders on the sample rack. The rack is then loaded onto the carousel.

1. Barcode and Host Query Mode
   - Non-interfaced: From SPECIMEN PROCESSING, select ADD PATIENT, enter the barcode number and schedule test requests. Specimen ID is the only required field.
   - Interfaced - Barcode: If interfaced to a host computer system, select DOWNLOAD from SPECIMEN PROCESSING. A message box will display the number of records received.

- Interfaced - Host Query: Sample identification and assay schedules are downloaded as the barcodes are read.

Place barcoded samples in the sample rack beginning with the first available carousel position. The barcoded samples can be loaded in any order. Sequence numbers will be assigned when the sample barcode is read just prior to sampling.

2. Non-Barcode Mode
   - Non-Interfaced: From SPECIMEN PROCESSING, select ADD PATIENT, enter the patient identification and schedule test requests. Specimen ID is the only required field.
   - Interfaced: If interfaced to a host computer system, select DOWNLOAD from SPECIMEN PROCESSING. A message box will display the number of records received.

Print a worklist and place specimens in the carousel as shown on the worklist.

B. Assay Procedure

1. Add a sufficient quantity of AIA-PACK PA test cup trays to the sorter drawer.
2. Load samples into carousel as instructed above. Select ASSAY START from the Main Menu and follow the instructions on the screen.

Procedural Notes

1. Lyophilized Substrate must be completely dissolved.
2. Ligand assays performed by the TOSOH AIA Systems require that the laboratory use water designated by the College of American Pathologists as Class I or by NCCLS as Type I. Water should be tested at least once per month and should be free of particulate matter including bacteria. The pH of the water should also be routinely tested. For further information, consult the NCCLS document "Preparation and Testing of Reagent Water in the Clinical Laboratory," NCCLS Document C3-A2, Volume 11 No. 13, originally approved as a guideline by NCCLS in August 1991.
3. If a serum specimen Prostate Specific Antigen concentration is found to be greater than the linearity limit of the assay, the specimen should be diluted with the PA Sample Diluting Solution and reassayed according to the Assay Procedure. The recommended dilution for samples containing greater than 100 ng/mL is 1:10 or 1:100. It is desirable to dilute the serum sample so that the diluted sample reads between 2 and 100 ng/mL. The dilution factor should be entered into the software. For further information on the dilution of specimens, refer to the AIA System Operator's Manual.
4. The AIA systems can store two different calibration curves for each analyte at one time. Therefore, up to two different lots of AIA-PACK PA Test cups can be used during the same run.

5. If the assay specifications for this test are not already in the system software, the specifications must be entered under test code 012.

Calculation of Results

The AIA Systems perform all sample and reagent handling operations automatically. The AIA Systems read the rate of fluorescence produced by the reaction and automatically convert the rate to Prostate Specific Antigen concentration in ng/mL.

For samples requiring dilution, the AIA Nex·IA will automatically perform dilutions and calculate results if the dilution factors are entered into the software. Dilution factors may be entered into the Test File, or pre-defined dilution factors may be selected in Specimen Processing.

Evaluation of Results

Quality Control

In order to monitor and evaluate the precision of the analytical performance, it is recommended that commercially available control samples be assayed daily.

If one or more control sample value(s) is out of the acceptable range, it will be necessary to investigate the validity of the calibration curve before reporting patient results.

Standard laboratory procedures should be followed in accordance with the regulatory agency under which the laboratory operates.

Limitations of the Procedure

For diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g., symptoms, results of other tests, clinical impressions, therapy, etc.).

Using AIA-PACK PA, the highest concentration of Prostate Specific Antigen measurable without dilution is 100 ng/mL, and the lowest measurable concentration is 0.05 ng/mL (assay sensitivity).

Although the approximate value of the highest calibrator is 50 ng/mL, the exact concentration may be slightly different.

Although hemolysis has an insignificant effect on the assay, hemolyzed samples may indicate mistreatment of a specimen prior to assay and results should be interpreted with caution.

Lipemia has an insignificant effect on the assay except in the case of gross lipemia where spatial interference may occur.

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show falsely elevated or decreased PSA values.

For a more complete understanding of the limitations of this procedure, please refer to the Specimen Collection and Handling, Warnings and Precautions, Storage and Stability, and Procedural Notes sections in this insert sheet.

Expected Values

Each laboratory should determine a reference interval which corresponds to the characteristics of the population being tested. As with all diagnostic procedures, clinical results must be interpreted with regard to concomitant medications administered.[10]

Results from the AIA-PACK PA assay should not be interpreted as being definitive for the presence or absence of prostatic cancer. Patients with levels of PSA within the reference interval found in apparently healthy subjects, may have prostatic cancer; patients with levels exceeding those in the reference interval may be prostate cancer free. Results from the AIA-PACK PA should be interpreted in the light of other clinical findings and diagnostic procedures such as DRE. Biopsy of the prostate is currently the medically accepted standard used to confirm the presence/absence of prostate cancer.

Reference Ranges

The interval given here was determined in serum samples from 863 apparently healthy male subjects.

Category	**Men**
Number of Samples (n)	863
Reference Interval	0 - 4.0 ng/mL

In this study, 98.7% of the healthy subjects had serum PSA concentrations less than or equal to 4.0 ng/mL. Results of this study are categorized below:

Category	Women	Men <40 years	Men ≥ 40 years
Number of Samples (n)	304	282	581
Mean (x)	0.0 ng/mL	0.63 ng/mL	1.08 ng/mL
SD	0.0 ng/mL	0.44 ng/mL	0.93 ng/mL
Range of Values	0.00 - 0.28 ng/mL	0.00 - 1.51 ng/mL	0.00 - 6.40 ng/mL
± 2 SD range		0.00 - 1.51 ng/mL	0.00 - 2.94 ng/mL

Expected Values for Management of Patients with Prostatic Cancer

Distribution of Serum AIA-PACK PA Concentrations in Healthy Subjects, Malignant and Benign Disease States

	Number of patients	0 - 4.0 ng/mL	4.01- 10.0 ng/mL	10.01- 20.0 ng/mL	20.01- 40.0 ng/mL	>40.0 ng/mL
Healthy Subjects						
Men<40	282	99.6%	0.4%	0%	0%	0%
Men≥40	581	98.3%	1.7%	0%	0%	0%
Total Men	863	98.7%	1.3%	0%	0%	0%
Women	304	100.0%	0.0%	0%	0%	0%
Total	1167	99.1%	0.9%	0%	0%	0%
Malignant Diseases						
Prostate						
Stage A	120	62.5%	23.3%	10.8%	2.5%	0.8%
Stage B	159	49.1%	22.0%	17.6%	6.3%	5.0%
Stage C	136	39.7%	17.6%	14.0%	16.2%	12.5%
Stage D	167	21.6%	8.4%	8.4%	11.4%	50.3%
Total Prostate	582	41.8%	17.4%	12.7%	9.3%	18.8%
Gastrointestinal Males	41	92.7%	2.4%	0.0%	2.4%	2.4%
Gastrointestinal Females	21	100.0%	0.0%	0.0%	0.0%	0.0%
Genitourinary Males	64	87.5%	10.9%	1.6%	0.0%	0.0%
Genitourinary Females	21	100.0%	0.0%	0.0%	0.0%	0.0%
Mammary Males	1	0.0%	100.0%	0.0%	0.0%	0.0%
Mammary Females	60	100.0%	0.0%	0.0%	0.0%	0.0%
Pulmonary Males	37	91.9%	8.1%	0.0%	0.0%	0.0%
Pulmonary Females	24	100.0%	0.0%	0.0%	0.0%	0.0%
Other Males[A]	17	100.0%	0.0%	0.0%	0.0%	0.0%
Other Females[B]	5	100.0%	0.0%	0.0%	0.0%	0.0%
Nonmalignant Diseases						
Benign Prostatic Hypertrophy Males	173	64.7%	23.7%	8.7%	2.3%	0.0%
Misc. Benign Genitourinary Males	23	82.6%	17.4%	0.0%	0.0%	0.5%
Misc. Benign Genitourinary Females	25	100.0%	0.0%	0.0%	0.0%	0.0%
Other Benign Males[C]	32	90.6%	9.4%	0.0%	0.0%	0.0%
Other Benign Females[D]	39	100.0%	0.0%	0.0%	0.0%	0.0%

2332 single specimens (patients) were analyzed for this distribution chart.

A. leukemia 1, parotid CA 1, liposarcoma and adrenal adenoma 1, kidney 2, testicular 6, pancreas 3, liver 3

B. pancreas 2, liver 2, ovarian 1

C. Pancreatitis 2,hepatitis 2,cirrhosis 4,asthma 1,cysts,mole,benign tumors (lung, ulcer, bowel)

D. pancreatitis 4, benign breast 4, hepatitis 4, cirrhosis 2, bronchitis 1, asthma 1, COPD 3, laryngitis, cysts, lipoma, thyroid adenoma, hyperthyroidism, hemangioma, benign tumors and colon, lung abscess, bronchitis, colitis, cholecystitis

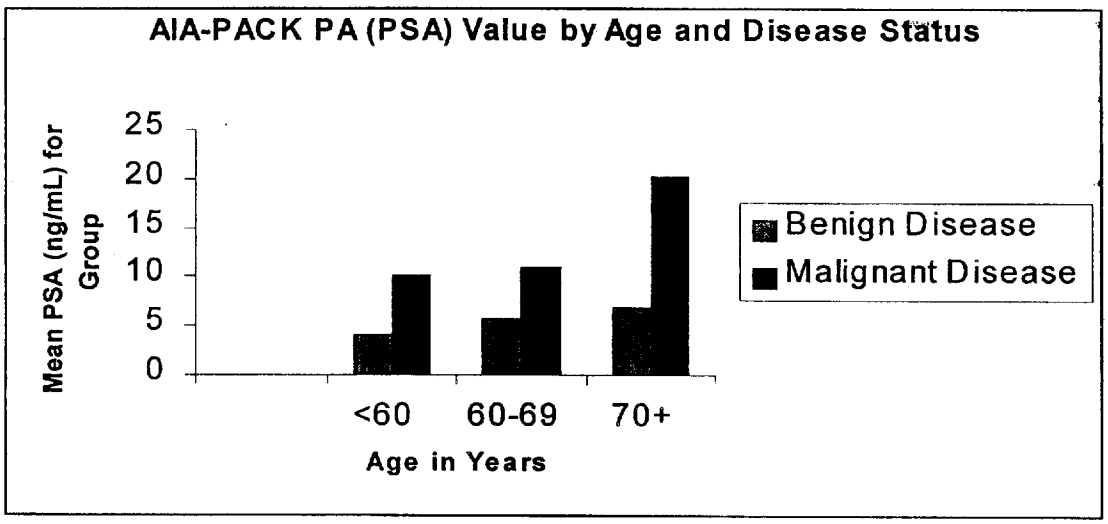
Expected Values for Detection of Prostate Cancer

Catalona, et al. (11) conducted a multi-center, prospective clinical trial on men over the age of fifty years to test the effectiveness of PSA along with digital rectal examination (DRE) in detecting prostate cancer. This study showed that PSA measurements in conjunction with DRE were more effective in detecting prostate cancer than either DRE or PSA measurements alone. PSA elevations above 4.0 ng/mL detected 41% of cancers that DRE did not find. Conversely, DRE detected 21% of cancers that PSA using a cutoff of 4.0 ng/mL did not uncover.

Our studies with the AIA-PACK PA reached similar conclusions: PSA testing should be done in conjunction with DRE because used in tandem these tests detect a far greater number of prostate cancers than either test used alone.

Retrospective Study

Retrospectively collected samples were obtained from 624 men between the ages of 50 and 90 (median age=68; mean = 68.8) on whom prostate biopsies were performed within thirty days after sample collection. Ninety-five percent of all AIA-PACK PA values were between 0.31 ng/mL and 105.5 ng/mL. The median value was 5.2 ng/mL. Patients whose PSA values fell out of the central 95% (33) were excluded from further analysis leaving a total of 591 samples. The chart below shows the mean value of PSA by age-category-disease status. The results indicate a significant association between biopsy result, age and PSA value.



Of the 591 patients in this retrospective study, DRE results were available on 567 (95.9%). The following chart shows the distribution of disease by DRE result. Only 58 of the 153 Prostate Cancer patients were identified by DRE alone. The predictive value of a suspicious DRE result in this study is calculated to be 24.2%.

Distribution of Disease Status by DRE Result (Retrospective Study)

DRE Result	Biopsy – Malignant	Biopsy – Benign	Total Numbers
Suspicious	58	182	240
Normal	95	232	327
Total	153	414	567

Distribution of Disease Status by PSA and DRE Result (Retrospective Study)

DRE Result	PSA (ng/mL)	Biopsy – Malignant	Biopsy – Benign
Suspicious	≥4.0	45	75
Suspicious	<4.0	13	107
Normal	≥ 4.0	73	173
Normal	<4.0	22	59

When used without DRE and age, PSA at a cutoff of 4.0 ng/mL yielded a positive predictive value of 32.2%. For this same group, DRE alone showed a positive predictive value of 24.2%. Using AIA-PACK PA (PSA) at a cutoff of 4.0 ng/mL with the DRE increased the number of correctly identified patients to 131. The positive predictive value using both tests in tandem was 27.0%. The added value of PSA and DRE over DRE alone is 47.7% ((131-58)/153).

Prospective Study

A second study, prospective in nature, included 523 men 50 years of age and older from 11 clinical sites referred to a urologist for determination of the presence of prostate cancer with no previous history of either prostate disease or evaluation for prostate cancer. Ninety-five percent of the values of PSA as measured by the AIA-PACK PA fell between 0.33 and 87.9 ng/mL. These central 95% patients (515) were used for further

statistical analysis. This study also showed the significant association between biopsy result and PSA value as measured by AIA-PACK PA. The following chart shows the distribution of disease by DRE result. Only 60 of the 187 Prostate Cancer patients were identified by DRE alone. The positive predictive value of a suspicious DRE in this study was 58.3%.

Distribution of Disease Status by DRE Result (Prospective Study)

DRE Result	Biopsy - Malignant	Biopsy – Benign	Total Numbers
Suspicious	60	43	103
Normal	127	285	412
Total	187	328	515

Distribution of Disease Status by PSA and DRE Result (Prospective Study)

DRE Result	PSA (ng/mL)	Biopsy – Malignant	Biopsy – Benign
Suspicious	≥4.0	48	28
Suspicious	<4.0	12	15
Normal	≥ 4.0	107	214
Normal	<4.0	20	71

When used without DRE and age, PSA at a cutoff of 4.0 ng/mL yielded a positive predictive value of 39.0%. Using AIA-PACK PA (PSA) at a cutoff of 4.0 ng/mL with the DRE increased the number of correctly identified patients to 167. The positive predictive value using both tests in tandem was 39.4%. The added value of PSA and DRE over DRE alone is 57.2% ((167-60)/187).

Of the 187 prostate cancers confirmed by biopsy in the above prospective study, Gleason grade scores ranged from 3 to 10. The median Gleason grade was 6.0 with a mean of 6.3. The mean value of PSA for men with a Gleason grade of 4 or less is lower than either those graded 5 – 7 or those grouped from 8 – 10 (5.7 ng/mL vs 10.3 ng/mL vs 20.1ng/mL).

Combined Studies

Combining the results of the prospective and retrospective studies detailed above, the results shown below were generated. Using DRE alone, 118 of the 340 patients with a malignant biopsy result (34.6%) would have been detected. Positive predictive value was determined to be 34.4%.

Distribution of Disease Status by DRE Result (Combined Study)

DRE Result	Biopsy - Malignant	Biopsy – Benign	Total Numbers
Suspicious	118	225	343
Normal	222	517	739
Total	340	742	1082

Distribution of Disease Status by PSA and DRE Result (Combined Study)

DRE Result	PSA (ng/mL)	Biopsy – Malignant	Biopsy – Benign	TOTALS
Suspicious	≥4.0	93	103	196
Suspicious	<4.0	25	122	147
Normal	≥ 4.0	180	387	567
Normal	<4.0	42	130	172
TOTALS		340	742	1082

When used without DRE and age, PSA at a cutoff of 4.0 ng/mL yielded a positive predictive value of 35.8%. Using AIA-PACK PA (PSA) at a cutoff of 4.0 ng/mL with the DRE increased the number of correctly identified patients to 298. The positive predictive value using DRE and PSA in tandem was 32.7%. The added value of PSA and DRE over DRE alone is 52.9% ((298-118)/340).

Note: Though our clinical studies clearly indicate that PSA and DRE together are useful in detecting a greater number of prostate cancers, negative results on either DRE or PSA or both do not preclude the presence of prostate cancer. Also, positive results on DRE and/or PSA do not confirm a diagnosis of prostate cancer. The results of a prostatic biopsy must be used to confirm a prostate cancer diagnosis.

Performance Characteristics

The following performance characteristics were determined using the TOSOH AIA-1200 Automated Immunoassay Analyzer. The AIA Nex·IA demonstrates equivalent performance.

Accuracy

a. **Recovery:** Three serum pools were spiked with four different levels of PSA and assayed before and after spiking.

Sample	Initial Value (ng/mL)	PSA Added (ng/mL)	Expected Value (ng/mL)	Measured Value (ng/mL)	Percent Recovery (%)
Serum A	19.95	75.69	95.64	90.59	94.7
	19.95	56.76	76.71	74.77	97.5
	19.95	37.84	57.59	59.96	103.8
	19.95	18.92	37.87	38.92	100.1
Serum B	7.20	75.69	82.88	83.26	100.5
	7.20	56.76	63.96	60.94	95.3
	7.20	37.84	45.04	46.30	102.8
	7.20	18.92	26.12	25.81	98.8
Serum C	1.00	75.69	76.68	75.65	98.7
	1.00	56.76	57.76	55.82	96.6
	1.00	37.84	38.84	38.85	100.0
	1.00	18.92	19.92	21.23	106.6

b. **Dilution:** Three serum samples containing high concentrations of PSA were serially diluted with PA Sample Diluting Solution and assayed.

Sample	Dilution Factor	Expected Value (ng/mL)	Measured Value (ng/mL)	Percent Recovery (%)
Serum A	none		89.0	100.0
	1:2	44.5	43.9	98.7
	1:3	29.7	30.6	103.2
	1:4	22.3	22.0	98.8
	1:10	8.9	8.9	100.3
	1:20	4.5	4.7	104.8
Serum B	none		78.2	100.0
	1:2	39.1	38.6	98.6
	1:3	26.1	25.8	98.7
	1:4	19.6	20.0	102.1
	1:10	7.8	7.9	101.1
	1:20	3.9	3.9	99.6
Serum C	none		95.4	100.0
	1:2	47.7	49.0	102.8
	1:3	31.8	32.2	101.3
	1:4	23.9	25.1	105.2
	1:10	9.5	10.0	104.9
	1:20	4.8	5.0	103.9

Precision

a. The intra-assay (within run) precision coefficient of variation was evaluated in four serum pools by 20 replicate determinations.

Intra-assay Precision

Sample	Number of Replicates	Mean (ng/mL)	Standard Deviation (ng/mL)	Coefficient of Variation (%)
Serum A	20	2.50	0.086	3.4
Serum B	20	20.86	0.371	1.8
Serum C	20	51.34	1.083	2.1
Serum D	20	75.69	2.776	3.7

b. The inter-assay (between run) precision coefficient of variation was evaluated at four different concentrations by analyzing samples in 20 separate runs.

Inter-assay Precision

Sample	Number of Replicates	Mean (ng/mL)	Standard Deviation (ng/mL)	Coefficient of Variation (%)
Serum A	20	2.35	0.112	4.8
Serum B	20	21.23	1.035	4.9
Serum C	20	50.87	1.841	3.6
Serum D	20	76.72	3.778	4.9

Specificity

The following substances were tested for cross-reactivity. The cross-reactivity (%) is the percent of the compound which will be identified as PSA. If these compounds are present in the specimen at the same concentration as PSA, the final result will be increased by these percentages.

Compound	Cross-reactivity (%)
PAP	0.0

Sensitivity

The minimal detectable concentration (MDC) of Prostate Specific Antigen is estimated to be 0.05 ng/mL. The MDC is defined as that concentration of PSA which corresponds to the rate of fluorescence that is two standard deviations from the mean rate of fluorescence of 20 replicate determinations of a zero calibrator.

Interference

Interference is defined, for purposes of this study, to be recovery outside of 10% of the known specimen mean concentration.

- Added hemoglobin (up to 473 mg/dL) and bilirubin (up to 17.8 mg/dL) do not interfere with the assay.
- Lipemia, as indicated by added triglyceride (up to 1,667 mg/dL), does not

interfere with the assay.

- Protein, as indicated by added albumin (up to 11.6 g/dL), does not interfere with the assay.
- Chemotherapeutic agents: Therapeutic concentrations of the following chemotherapeutic agents added to serum samples which were then assayed, do no interfere with AIA-PACK PA Assay: Cisplatin, Bleomycin, Adriamycin, Cytoxan, Vincristine Diethylstilbestrol, Methotrexate, 5-Fluorouracil and Mitomycin C.
- A study of 53 men with no prior evidence of prostate disease who were regularly taking aspirin, anticoagulants, anti-hypertensives, anti-inflamatory drugs, antibiotics, hypo-glycemics and/or anti-arrhythmic medication was undertaken to determine whether these commonly prescribed medications would affect the blood levels of PSA. Levels of PSA as measured by the AIA-PACK PA were not affected by the use of these drugs in a prostate disease-free cohort.

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AIA-PACK
PA Calibrator Set

Intended Use

The AIA-PACK PA Calibrator Set is intended for IN VITRO DIAGNOSTIC USE ONLY for the calibration of the AIA-PACK PA Assay.

Summary and Explanation

The AIA-PACK PA Calibrator Set contains human serum with assigned levels of Prostate Specific Antigen. Calibration should be performed according to the schedule indicated in the AIA System Operator's Manual. The calibrators in this set are prepared gravimetrically and compared to internal reference standards.

Material Provided (Cat. No. 020363)

2 x 1 mL	Zero Calibrator Human serum containing no detectable concentration of PSA (0 ng PSA/mL), and 0.1% sodium azide as preservative.
2 x 1 mL	Positive Calibrator Human serum containing the assigned concentration of PSA (approximately 50 ng PSA/mL, described on each vial) and 0.1% sodium azide as preservative.

Warnings and Precautions

- The AIA-PACK PA Calibrator Set is for in vitro diagnostic use.
- These materials contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Human sera used in the preparation of this product has been tested by FDA approved methods and found negative for the presence of HBsAg and antibody to HIV-1 and HCV. Because no test method can offer complete assurance that products derived from human blood will not transmit infectious agents, it is recommended that this product be handled with the same precautions as used for patient samples.
- Do not use beyond the expiration date.

Preparation and Storage

- The AIA-PACK PA calibrators are provided ready to use.
- Bring calibrator to room temperature (18° - 25° C) for use.
- Always store the Calibrator Set in an upright position at 2° - 8° C when not in use.

Stability

When stored unopened and refrigerated at 2° - 8° C, the AIA-PACK PA Calibrator Set is stable until the expiration date on the label. After opening, the calibrators should be used within 24 hours.

Procedure

Refer to the CALIBRATION PROCEDURE in the AIA-PACK section of this analyte application. For additional procedural instructions regarding calibration, refer to the AIA System Operator's Manual.

1. When using new calibrator lots, enter the calibrator concentration values and lot number in the AIA Nex·IA test file (refer to the AIA System Operator's Manual for details).
2. Load the appropriate amount of AIA-PACK PA test cups in the sorter drawer.
3. Select CALIBRATION REQUEST. Enter the 16-digit calibrator lot number.
4. Add the appropriate amounts of each calibrator to sample cups (refer to the instrument worksheet for the sample volume.)
5. Print a worklist and place the sample cups in the carousel position indicated.
6. Select START. Verify that the carousel positions on the worklist match the starting carousel position on the screen.

Assignment of Values

The AIA-PACK PA Calibrator Set contains assigned concentrations of Prostate Specific Antigen. The assigned value is determined on a lot-to-lot basis and is designed to provide an assay calibration range of approximately 0.0 to 100.0 ng/mL of Prostate Specific Antigen. The calibrators in this set are prepared gravimetrically and compared to internal reference standards.

Results

- The mean rate for the zero calibrator should be <3.0 nM/sec.
- Since there is a direct relationship between concentration and rate, the rates should increase as the concentration increases.
- The replicate values should be within a 10% range.

Limitations

The AIA-PACK PA Calibrator Set is designed solely for use with AIA-PACK assay procedures.

References

1. AIA Nex·IA Analyte Application Manual. Tosoh Medics, Inc., South San Francisco, CA.
2. AIA Nex·IA Operator's Manual. Tosoh Medics, Inc., South San Francisco, CA.

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AIA-PACK
PA Sample Diluting Solution

Intended Use

The AIA-PACK PA Sample Diluting Solution is intended for IN VITRO DIAGNOSTIC USE ONLY to dilute patient samples that have concentrations of Prostate Specific Antigen above the linear range of the assay.

Summary and Explanation

The AIA-PACK PA Sample Diluting Solution contains human serum with no detectable concentration of Prostate Specific Antigen (PSA). This Sample Diluting Solution is to be used only with samples that are being tested for Prostate Specific Antigen concentrations using the AIA-PACK PA assay.

Material Provided (Cat. No. 020563)

4 x 4 mL Sample Diluting Solution

Human serum containing no detectable concentration of Prostate Specific Antigen (0 ng PSA/mL), and 0.1% sodium azide as a preservative.

Warnings and Precautions

- The AIA-PACK PA Sample Diluting Solution is for in vitro diagnostic use.
- These materials contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Human sera used in the preparation of this product has been tested by FDA approved methods and found negative for the presence of HBsAg and antibody to HIV-1 and HCV. Because no test method can offer complete assurance that products derived from human blood will not transmit infectious agents, it is recommended that this product be handled with the same precautions as used for patient samples.
- Do not use beyond the expiration date.

Preparation and Storage

- The AIA-PACK PA Sample Diluting Solution is provided ready to use.
- Always store the Sample Diluting Solution in an upright position at 2° - 8° C when not in use.

Stability

When stored unopened and refrigerated at 2° - 8° C, the AIA-PACK PA Sample Diluting Solution is stable until the expiration date on the label. After opening, the Sample Diluting Solution is stable for up to 90 days when refrigerated at 2° - 8° C.

Procedure

Refer to the AIA System Operator's Manual for additional procedural instructions regarding sample dilution.

1. If a specimen is found to contain greater than the linearity limit of 100 ng/mL, the specimen should be diluted with the Sample Diluting Solution and assayed according to the procedure in the AIA-PACK section of the analyte application.
2. The AIA Nex·IA will perform dilutions automatically if the dilution factors are entered into the software prior to assaying the diluted sample.
3. The recommended dilution for serum containing greater than 100 ng/mL is 1:10 or 1:100. However, it is desirable to dilute the serum samples that contain more than 100 ng PSA/mL so that the diluted sample reads between 2 and 100 ng PSA/mL.

Results

- When an auto-dilution is performed, the AIA Nex·IA will calculate the final result.

Limitations

The AIA-PACK PA Sample Diluting Solution is designed solely for use with AIA-PACK assay procedures.

References

1. AIA Nex·IA Analyte Application Manual. Tosoh Medics, Inc., South San Francisco, CA.
2. AIA Nex·IA Operator's Manual. Tosoh Medics, Inc., South San Francisco, CA.

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AIA-PACK
PA Calibration Verification/Linearity Test Set

Intended Use

The AIA-PACK PA Calibration Verification/Linearity Test Set is intended for IN VITRO DIAGNOSTIC USE ONLY for the verification of the calibration and linearity of the AIA-PACK PA Assay.

Summary and Explanation

The AIA-PACK PA Calibration Verification/Linearity Test Set can be used as part of a quality assurance program to assist in complying with various regulatory requirements under which an institution may operate. Specific guidelines for the acceptability of data are determined by the institution in conformance with these requirements. Good laboratory practices stipulate using different materials for calibration and quality control purposes. Calibration Verification Materials should be analyzed as unknown test samples according to the directions stated in the AIA System Operator's Manual.

Material Provided (Cat. No. 020663)

2 x 4 mL	Sample Diluting Solution (SDS) Human serum containing no detectable concentration of PSA (0 ng PSA/mL), and 0.1% sodium azide as preservative.
2 x 2 mL	Calibration Verification Material (CVM) Human serum containing the assigned concentration of PSA (approximate upper linearity limit of 100 ng PSA/mL, described on each vial) and 0.1% sodium azide as preservative.

Warnings and Precautions

- The AIA-PACK PA Calibration Verification/Linearity Test Set is for in vitro diagnostic use.
- These materials contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such materials, always flush with large volumes of water to prevent azide build-up.

- Human sera used in the preparation of this product has been tested by FDA approved methods and found negative for the presence of HBsAg and antibody to HIV-1 and HCV. Because no test method can offer complete assurance that products derived from human blood will not transmit infectious agents, it is recommended that this product be handled with the same precautions as used for patient samples.
- Do not use beyond the expiration date.

Preparation and Storage

- The Calibration Verification Material and Sample Diluting Solution are provided ready to use.
- The materials should be at room temperature (18° - 25° C) prior to use.
- Always store the Calibration Verification/Linearity Test Set in an upright position at 2° - 8° C when not in use.

Stability

When stored unopened and refrigerated at 2° - 8° C, the AIA-PACK PA Calibration Verification/Linearity Test Set is stable until the expiration date on the label. After opening, the materials should be used within 24 hours.

Procedure

The frequency of performing calibration verification and linearity of the AIA-PACK PA assay is established by each institution in accordance with its quality assurance program and appropriate regulatory requirements. Refer to the ASSAY PROCEDURE in the AIA-PACK section of this analyte application. For additional procedural instructions, refer to the AIA System Operator's Manual.

1. Make the desired dilutions of the Calibration Verification Material with Sample Diluting Solution and mix well. A sufficient amount should be made to assay each diluted sample in triplicate.
2. Load the appropriate amount of AIA-PACK PA test cups on the instrument.
3. Program the instrument to run each of the prepared dilutions three times.
4. Add the appropriate amounts of each diluted sample to sample cups (refer to the instrument worksheet for the amount required in each sample cup).
5. Place the sample cups in the sample rack on the instrument.
6. Start the assay.
7. Record the values obtained on the appropriate forms and calculate the desired statistics.

Assignment of Values

The Calibration Verification Material contains an assigned concentration of Prostate Specific Antigen. The assigned value is determined on a lot-to-lot basis and is designed to approximate the upper linear range of the assay. The Calibration Verification Material in this set are prepared gravimetrically and compared to internal reference standards.

Results

- Make a determination of the acceptability of the data according to the specific guidelines established by your institution which satisfy the regulatory requirements under which your institution operates. If results do not meet your specifications, please initiate corrective action, as appropriate, or contact Tosoh Medics, Inc. for assistance.
- Retain test records in the laboratory in the designated location for future reference.

Limitations

The AIA-PACK PA Calibration Verification/Linearity Test Set is designed solely for use with AIA-PACK assay procedures. The AIA-PACK PA Calibration Verification/Linearity Test Set will only verify linearity between the established sensitivity of the assay and the value of analyte level listed on the Calibration Verification Material label.

References

1. AIA Nex·IA Analyte Application Manual. Tosoh Medics, Inc., South San Francisco, CA.
2. AIA Nex·IA Operator's Manual. Tosoh Medics, Inc., South San Francisco, CA.

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